APPLICANTS: Peled et al.
U.S.S.N.: 09/986,897

REMARKS

Upon entry of the amendment, claims 1-5, 7-8, 18-23, 25-30, 32-34, 36, 42-48 and 51-62 will be pending in the application. Claims 6, 9-17, 24, 31, 35, 37-41 and 49-50 are cancelled with the present amendment and new claims 51-62 added. Claims 1, 4, 5, 7, 10, 11, 12, 37, 39, 42, 43, and 44 are amended. Support for the amendments to claim 1 appears in, e.g., now cancelled claims 17 and in the specification at page 6, lines 5-15, and the paragraph bridging pages 32-33. New claims 51-62 are supported in the specification at, e.g., the Examples section of the specification, and on page 26, lines 3-6. The remaining amendments more clearly point out the subject matter claimed, address various informalities, and/or clarify antecedent bases.

No new matter is added by these amendments. The cancellation of claimed subject matter of does not constitute an admission by Applicants that the subject matter no longer claimed is not patentable. Applicants reserve the right to pursue all cancelled subject matter in a continuing application or applications.

Priority Benefit Under 35 U.S.C. § 120

The Examiner has asked that Applicants provide a detailed analysis demonstrating that the instant claims are entitled to the benefit of all the parent application filing dates. The Examiner in particular states that the "subject matter claimed in claims 6, 8, 18, 33 and 43, that is hematopoietic cells, wherein hematopoetic cells are obtained from neonatal umbilical cord blood and wherein transition metal chelator is tetraethylpentamine does not have support in the parent applications Serial Numbers: 09/161,659 ("the '659 application"), 09/130,367 ("the '367 application", and 09/024,195 ("the '195 application"). Applicants provide this analysis below.

In the '195 application, hematopoietic cells are described and the specification teaches that the hematopoietic cells may be obtained from, amongst others, neonatal umbilical cord blood (see page 9, lines 13-14; claim 20) and that a transition metal chelator may be tetraethylenepentamine (see page 7, line 12). Accordingly, the '195 specification discloses the subject matter claimed in claims 6, 8, 18, 33 and 43 of the present application.

The '367 application also teaches hematopoietic cells and further teaches that the hematopoietic cells may be obtained from, amongst others, neonatol umbilical cord blood (page

10, lines 5-6; claim 20) and that a transition metal chelator may be tetraethylenepentamine (see page 8, line 21). Accordingly, the '367 specification discloses the subject matter claimed in claims 6, 8, 18, 33 and 43 of the present application.

The '659 application application refers to co-pending applications 09/130,367 and 09/024,195 as teaching the transition metal chelating agent tetraethylpentamine (see page 6, lines 4-9) and further teaches the use of hematopoietic cells (see page 9, line 10; page 17, line 8-9) and that such cells may be obtained form neonatal umbilical cord blood (see page 9, lines 15-16; page 17, lines 14-15). Accordingly, the '659 specification also discloses the subject matter claimed in claims 6, 8, 18, 33 and 43 of the present application.

In view of the above, Applicants respectfully submit that the claimed subject matter in the present application has clear support in the '195, '367 and '659 parent applications.

Rejections under 35 U.S.C. 112, First Paragraph

Claims 1-8, 15-33, and 40-49 are rejected for overbreadth. Claims 6, 9-17, 24, 31, 35, 37-41 and 49-50 are cancelled. The rejection is traversed to the extent it is applied to the remaining claims as amended.

Independent claims 1 and 25, from which the remaining claim subject to the rejection depend, have been amended so that they are drawn to an expanded hematopoietic cell population. The Examiner acknowledges that the specification enables claims drawn to $CD34^+$ cell populations but contends that the specification is not enabled for any cells (*See*, Office Action at page 3).

Applicants respectfully submit that the full breadth of the invention now claimed can be practiced using the knowledge available to one of ordinary skill in the art when coupled with the teachings of the specification. For example, the specification teaches that transition metal chelators are effective in enhancing expansion and inhibiting differentiation of hematopoietic cell types in addition to those that are CD34+ selected. Pages 37, line 10 to page 38, line 10, and Table 2 of Example 1 of the specification teaches that transition metal chelators stimulate expansion while inhibiting differentiation of murine erythroleukemia cell cultures as well as undifferentiated CD34+ cells. Other examples of growth induction and inhibition of cell

differentiation by transition metal chelators in diverse cell populations at various stages of differentiation are also taught in the specification (*See*, for example, Table 2- Effect of TEPA on murine erythroleukemia cells; Figure 5, effect of TEPA on erythroid precursors form peripheral blood mononucleocytes; Example 4, Table 4, Effect of TEPA on embryonal stem cells; and page 48, Example 4- Effect of TEPA on hepatocyte growth and differentiation).

The specification additionally teaches that hematopoietic reconstitution of lethally irradiated mice with bone marrow cells expanded *ex vivo* with the transition metal chelator TEPA and cytokines results in superior WBC recovery and survival as compared to cells expanded with cytokines alone (*See*, Example 5, pages 51 and 52, and Table 7).

Thus, contrary to the Examiner's contention, the specification discloses methods for *ex vivo* expansion of and inhibition of differentiation of stem/progenitor cells from a broad range of cells in addition to CD34+ cells. The instant specification is clearly enabling for expanded hematopoietic cells in addition to those that are CD34+, or cells selected on the basis that they are CD34+. The specification fulfills the requirement for "a recitation of a representative number of cell types falling within the scope of the (claimed) genus", as indicated by the Examiner (*See* Office Action at page 5, paragraph 1).

The teachings of the specification have also been used to practice the claimed invention for hematopoietic cells that are not CD34+ selected. One example is illustrated in the attached Declaration of Eitan Fibach[1] ("the Fibach Declaration"), which discusses the use of the claimed methods on AC133 cells. AC133 cells have high self-renewal capability, maintain early hematopoietic stem/progenitor cell (HSPC) characteristics, and show superior survival in culture, as compared to CD34+ cells.

The Fibach Declaration demonstrates that following a three week large-scale clinical grade expansion, the yield of cord blood-derived early progenitor cells (TNC, CFUc, CD34+ cells and CD34+CD38- cells) in high affinity transition metal chelator-supplemented (5 μM TEPA) culture initiated with AC133+ cells was statistically similar with that initiated with a same number of CD34+ cells. In addition, similar proportions of cells expressing myeloid,

[1] The Declaration has been prepared for a response in corresponding application U.S.S.N. 09/463,320.

lymphoid and megakaryocytic phenotype were found in cultures initiated either with CD34+ or AC133+ cells.

Thus, in addition to CD34+ cells, the claimed methods can be used on the additional cell types disclosed in the specification and AC 133+ cells cultured in the presence of a transition metal chelator which results in efficient expansion and inhibition of differentiation of hematopoietic stem/progenitor cells. These results clearly indicate that: (i) the methods of the present invention can be successfully extrapolated to populations of undifferentiated cells other than CD34+ cells; (ii) using the methods taught in the instant specification, one of ordinary skill in the art would expect, with a reasonable degree of success, to effectively expand and inhibit differentiation of a broad range of undifferentiated hematopoietic cells.

The Examiner has further stated that the present invention "must be considered unpredictable" since the mechanism of the effects of copper are as yet unknown. To the contrary, elucidation and knowledge of the mechanism of a drug's biological activity is hardly an acceptable criterion for predictability of the activity of the drug. Examples abound in which drugs are approved and routinely used for specific therapeutic effects, the pathways and mechanisms of which are poorly understood (aspirin and pain relief is only one example). Although understanding of the effects of copper on the process of differentiation will undoubtedly be of great value, demonstration of the predictable effects of transition metal chelators on expansion and inhibition of differentiation of hematopoietic cells, as taught in the instant specification, need not depend on such understanding. Further, the Examiner has stated that "the scope of the claims must bear a reasonable correlation with the scope of enablement". The examples brought hereinabove provide evidence for clear enablement for expansion and inhibition of differentiation of diverse types of undifferentiated cells to be found in the instant specification. Reconsideration and withdrawal of the rejection for overbreadth is respectfully requested.

Claims 1-8, 15-33 and 40-49 are also rejected for lack of written description. Claims 6, 9-17, 24, 31, 35, 37-41 and 49-50 are cancelled. The rejection is traversed to the extent it is applied to the claims as amended.

The claims have been amended so that they are no longer drawn to any cell population but are instead drawn to an expanded hematopoietic cell population (emphasis added). The Examiner acknowledges that Applicants were in possession of CD34+ cell populations cultured *ex vivo*. Applicants submit that the specification demonstrates that they were also in possession of the genus "hematopoietic cell population" when the application was filed. As described above, Applicants disclose in the specification that transition metal chelators promote proliferation while inhibiting differentiation of many hematopoietic, and non-hematopoietic, cell types. The cell types include, e.g., murine erythroleukemia cell cultures, erythroid precursors form peripheral blood mononucleocytes embryonal stem cells, and bone marrow cells from lethally irradiated mice expanded *ex vivo* hepatocytes. This demonstrates that Applicants were in full possession of the invention now claimed.

In view of the foregoing comments, Applicants submit the pending claims as amended meet the requirement for written description. Applicants respectfully request that the rejections under 35 USC 112, first paragraph be withdrawn.

Rejections under 35 U.S.C. 102(b)

Claims 1-5, 17-18, 24, 25-30, 42-43, and 49 are rejected as anticipated by Percival , J. Nutrition 122: 2424-2429 (1992) ("Percival"). Claims 17, 24, and 49 are cancelled. The rejection is traversed to the extent is applied to the claims as amended.

Claim 1, from which depend claims 2-5, has been amended to incorporate the subject matter of claim 6, which is not subject to the rejection. Similarly, independent claim 25, from which depends claims 26-30 and 42-43, has been amended to incorporate the subject matter of claim 31, which also is not subject to the rejection. Therefore, all of the claims as amended now are drawn to subject matter that was not subject to the rejection. Therefore, the rejection of claims 1-5, 17-18, 24, 25-30, 42-43, and 49 as anticipated by Percival can be withdrawn.

Claims 1-8, 15-17, 19-24, 25-33, 40-42, and 44-49 are rejected as anticipated by Cicuttine et al. Blood 80: 102-112 (1992) ("Cicuttine"). Claims 6, 15-17, 24, 31, 40-41 and 49 are

cancelled. The rejection is traversed to the extent it is applied to the remaining claims as amended.

Claim 1, from which depend claims 2-5, 7-8, 15-17 and 19-24, has been amended to specify the claimed expanded hematopoietic cell population is cultured in the presence of a transition metal chelator. Cicuttine does not describe this required feature of the claim and is silent about transition metal chelators. Therefore, Cicuttine does not describe the invention of claim 1, and the claims depending from claim 1.

Claim 25, from which the remaining claims subject to the rejection depend, requires an expanded hematopoietic cell population cultured *ex-vivo* in a culture medium where the hematopoietic cell population is expanded yet not further differentiated as compared to *ex-vivo* seeded cells from which said hematopoietic cell population originated.

Cicuttine does not describe this feature of the claimed invention. Applicants note the Examiner's statement that Cicuttine describes stimulation of growth and inhibition of differentiation of hematopoietic stem or progenitor cells by culturing the cells using defined growth conditions. The Examiner further states that since zinc has an affinity for copper, and would reduce copper utilization, and therefore culturing hematopoietic cells with zinc would inherently reduce a cell's capacity of utilizing copper. The Examiner further states that Cicuttine teaches cells isolated from cord blood and cells resuspended in PBS.

Applicants respectfully disagree. Cicuttine does not teach the use of transition metal chelators or reducing the capacity of hematopoietic cells in utilizing copper to inhibit differentiation during ex-vivo expansion, as taught by the claims of the present invention. Moroever, the cell line Ciccutini cultures in the presence of zinc are not hematopoietic cells but instead are stromal cell lines. This reference reports the transformation of stromal cells of human bone marrow origin with a SV40 large T antigen gene controlled by a Zinc-inducible metallothionein promoter.

Ciccutine describes the establishment of a human stromal cell line from bone marrow cells transformed with an SV40 large T antigen gene under control of a Zinc-responsive element of a metallothionein promoter (page 103, left column, third and fourth paragraph). The resultant stromal cell line (SCL) is then used to co-culture hematopoietic cells, upon inactivation of the

immortalizing T antigen gene by the removal of Zinc from the culture medium. Thus, addition or removal of Zinc from the culture medium is taught as a means of regulation of proliferation of the stromal cell culture only. In none of the examples described in Cicuttine is the co-culturing of hematopoietic cells performed in the presence of Zinc. Further, the Zinc-sensitive transformed stromal cell lines taught by Cicuttine does not exhibit any characteristic hematopoietic phenotype, and were negative for markers CD34, CD2, CD3, CD4, CD10, CD19, CD20, CD14 and CD15 (page 105, right column, first paragraph).

The Examiner goes on to assert that Ciccutine teaches that its method will result in growth of hematopoietic cells while inhibiting their differentiation. However, Cicuttine teaches that CD34+ cells could not be sustained for more than 12 days in culture without the SCL feeder layer (page 106, right column, second paragraph). CD34+ cord blood cells co-cultured with the Zinc-sensitive transformed SCL supported expansion of colony forming cells up to day 18 (page 106, right column, second paragraph). No inhibition of differentiation was demonstrated or implied.

Thus, the presence of a Zinc-sensitive promoter in the bone marrow stromal cell line transformed with SV40 T antigen gene taught by Cicuttine does not in any way anticipate the invention now claimed.

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On the basis of the foregoing amendments, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below. A petition for a three-month extension of time accompanies this response. The Commissioner is authorized to charge any fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Reference No. 24024-501 CON 2A.

Respectfully submitted,

FOR _____ Reg No. 41,874
Iver R. Elrifi, Reg. No. 39,529
Attorney for Applicants
c/o MINTZ, LEVIN
Tel: (617) 542-6000
Fax: (617) 542-2241
Customer No. 30623

Dated: December 2, 2003

TRA 1855172v3